

## CHIMERIC VIRUS-LIKE PARTICLE ANTIGEN PRESENTATION AND DELIVERY SYSTEM

The present invention relates to biologically useful particles. In particular it relates to modified particles derived from the yeast retrotransposon Ty. Particles formed from such proteins are immunogenic and can be used in immunotherapeutic or prophylactic vaccines or as diagnostic agents.

An ideal immunogen is a polymer of multiple antigen determinants assembled into a high molecular weight, particulate complex. A substantial disadvantage of most antigens produced by recombinant DNA techniques for vaccines is that they are usually produced as simple monomeric proteins. This is not the ideal configuration of an immunising antigen as it does not readily permit the cross-linking of the components of the immune system. Such crosslinking is required for maximum stimulations of humoral and cellular immunity. For these reasons it would be advantageous to develop polyvalent, particulate carrier systems for immunising antigens.

WO-A-8803562 and WO-A-8803563 describe the use of certain fusion proteins derived from retrotransposons or RNA retroviruses for pharmaceutical, diagnostic or purification applications. Such particles are designated virus-like particles (VLPs) when derived from the yeast retrotransposon Ty. The above published PCT applications note that polyvalent particles are useful for immunisation purposes because their polyvalent nature provides that more antibodies will be raised against the particulate antigens used. The particles are formed of fusion proteins having a particle-forming sequence and, in some embodiments at least, an antigenic sequence. In the examples, the antigenic sequence is positioned C-terminal to the particle-forming sequence.

While the above approach is promising, a potential difficulty is that insertion of the antigen at the C-terminal end of the particle-forming protein may not in all cases be optimal for presentation to the immune system. Animals immunised with recombinant VLPs may elicit a higher titre response to the Ty component than to the added antigen. It would therefore be highly advantageous to construct antigen-presenting particles where the antibody response to the added antigen is augmented. Such particles might also have enhanced ability to stimulate a cell-mediated immune response, such as a T-cell response, a Cytotoxic T-lymphocyte (CTL) response or a Natural Killer (NK) cell response. It would further be advantageous if, following immunisation with such particles, the antibody response to the particle-forming moiety was reduced or preferably prevented.

One way to improve the presentation of the antigenic sequence to the immune system might be to insert the antigenic sequence of interest within the particle-forming sequence. However, correct insertion of the antigenic site within the particle-forming protein is likely to be critical for particle formation. Insertions might disrupt the secondary and tertiary structure determinants of the monomer, or the quaternary interactions between monomers necessary for particle formation.

One approach to deduce suitable surface-exposed insertion sequences has been to use the understanding of the three-dimensional structure of viruses elucidated by X-ray crystallography. Such precise analysis of the structure of the polio virus has enabled particulate chimaeric proteins to be created whereby heterologous antigenic sequences are substituted for amino-acids present in the surface-exposed

epitopes of this virus (Dedieu et al., *J. Virol.* (1992) 66 3161-3167; Burke et al, *Nature* (1988) 332 81-82; Evans et al., *Nature* (1989) 339 385-388). However, these polio virus constructions are limited by the need to produce a viable virus; even some very short sequences cannot be tolerated.

Detailed analysis as described for poliovirus is not possible for proteins which have not yet been crystallised. Where particles have a well-characterised tertiary  $\beta$ -barrel structure, internal insertions of heterologous antigenic sequences into presumed surface exposed regions have been made using predictive models based on sequence alignment. For example, hybrid particles prepared from the hepatitis B core antigen and an antigen derived from a virus with an analogous secondary structure were found to maintain particle formation and enhance the immunogenicity of the inserted antigen (Schodel et al., *J. Virol.* (1992) 66 106-114; Brown et al., *Vaccine* 1991 9 595-601). Substitutions of heterologous peptides into presumed surface-exposed, immunodominant regions of the hepatitis B surface antigen also gave rise to particulate, chimaeric proteins with enhanced immunogenicity (von Brunn et al., *Vaccine* 1991 9 477-601), although considerable amounts of lipid were found to be associated.

However, retrotransposons have a very poorly understood structure and it is not currently believed that they possess a  $\beta$ -barrel (Bums et al., *J. Mol. Biol.* (1990) 261 207-211). Suitable sites for insertion of antigens into these particulate proteins are therefore not known or predictable. In retroviruses (which have a very similar structure to retrotransposons) it has been shown that insertion of an antigen into the middle of the gag sequence destroys the particle-forming nature of this sequence (Luo et al., *Proc Natl. Acad. Sci. USA* 89 10527-10531 (1992)).

The present inventors have identified the surface-exposed immunodominant epitopes within the yeast retrotransposon Ty p1. Immunogenic sites are not necessarily surface exposed; high titre antibodies are frequently elicited against core proteins during viral infections even though such proteins are not exposed on the surface of the particle (eg the influenza nucleoprotein). The inventors have also found that insertion of heterologous antigenic sequences into such epitopes does not prevent particle formation. In retrotransposons the size of insertion which can be tolerated without disrupting particle formation appears to be remarkably large; much greater than has been described for any other system, where generally substitutions have been preferred. The resulting hybrid particles exhibit reduced immunogenicity of the particle forming protein, and an enhanced immune response to the inserted sequence.

According to a first aspect of the invention, there is provided a non-natural particle-forming protein comprising a first self-assembling particle forming amino acid sequence substantially homologous with a yeast retrotransposon Ty p1 protein and a second amino acid sequence, wherein the second sequence is antigenic and is incorporated within an epitope of the first amino acid sequence, which epitope, on particles formed from the first amino-acid sequence alone, is surface-exposed.

Such constructions may be produced either by insertion of antigenic sequences into these surface epitopes to form true hybrid proteins, or by substitution of the native amino acids found at such sites with the amino acid sequence of interest, or by a combination of deletion, substitution and insertion.

The surface-expressed epitopes will generally be found in the N-terminal half of the first particle forming protein, the sequence of which is disclosed in Dobson et al., 1984